NOTES AND REVIEWS

Development of Flesh flies.—Kunkel (1918, Journ. Exp. Zool., 26:255-264) has tested the effects of mammalian thymus and thyroid on the development of flesh flies (Lucilia caesar and Lucilia sericata). When fed exclusively upon thyroid, growth of the larvae is slightly retarded; the resulting pupae are reduced in size, pupation is initiated earlier than normal and the period of pupation is shortened. Thymus tends to increase the size of the larvae. The results resemble those of similar experiments with vertebrates but are not so striking.

Terminology of Metamorphosis.-Comstock (1918, Ann. Ent. Soc. Am., 11:222-224) points out that in insects usually designated as having incomplete metamorphosis two distinct types of metamorphosis occur, one represented by such orders as Hemiptera and Orthoptera in which the development is direct, and the other represented by Plecoptera, Odonata, and Ephemerida in which there is cenogenetic development. The recognition of the distinct differences existing between the two groups of insects heretofore associated together gives support to a proposed revision of the following form: (1) Gradual Metamorphosis or paurometabolous development, characteristic of Orthoptera, Hemiptera, et al. (2) Incomplete Metamorphosis or hemimetabolous development, characteristic of Plecoptera. Odonata, and Ephemerida. (3) Complete Metamorphosis or homometabolous development, characteristic of Diptera, Lepidoptera, et al. Comstock proposes the restriction of the term nymph to the immature stages of gradual metamorphosis; the term naiad for the immature stages of Plecoptera, Odonata, and Ephemerida; and the term larva for the immature stages of all insects having complete metamorphosis.

PAUL S. WELCH.

Department of Zoology, University of Michigan.

> NOTES ON TECHNIQUE (Abstracted by Dr. V. A. Latham)

A Mounting Medium.—The best mounting medium is liquid petrolatum. It has the proper consistency for mounts, is less sticky, does not become acid as is so common with the usual Canada balsam

38

AMERICAN MICROSCOPICAL SOCIETY

of these times, does not require thinning with its solvent which os changes the refractive index and causes blood stains, such as that of Romanowsky, to fade. It has superior optical qualities. It is easily used for small insects and for sporangia of fungi, especially moulds. For permanent mounts the cover must be sealed with gold size or other cement. For the introduction of this liquid medium I believe we are indebted to Dr. Alfred C. Coles of England, who gave the method of using. ("Paraffin as an Oil Immersion Fluid," in *English Mechanic*, February 14, 1914). His work on *Spirochæta pallida* in the same journal for Dec. 1909, p. 267-8 and on the flagellæ of Bacteria, *idem*. Dec. 1909, page 308, will interest many.

Further Note on Mounting in Liquid Petroleum "Sea-cure" (English Mechanic, Jan. 17, 1919) suggests the following: Make the smear of bacteria or prepared Diatoms on thin glass. After drying, fix in 1/20 solution of carbolic acid before staining, if bacteria. Then stain, put on one drop of liquid petroleum, and use a cover glass and cedar oil for immersion. When the examination is finished wash off the petroleum by slipping the slide into a stoppered bottle of petrol. Keep the slide in a vertical-rack slide box. Number the slide and keep your notes in the box with it. This method is much easier for use with malarial slides in the tropics, and with preparations where the aniline stains have been used as these are not affected by petroleum in the way they are by the cedar oil. If microscopists would study Bacteria as they do Diatoms with dark ground illumination and a 1/12 oil immersion and with oblique light, some new results might be achieved for science.

Simplified Technic for Determination of Pale Spirochetae.—Quioc. (Paris Medical, p. 73, July 27, 1918, 8, No. 30; Abstract, J. A. M. A. p. 1616, Nov. 9, 1918) describes the superior and unfailing advantages of the Fontana-Tribondeau technic in the early or late differential diagnosis of syphilis. The organis debris and red cells are partially dissolved, while the pale spirochete is shown up clearly from other spirochetes. (T) Dissolve cold 1 gm. AgNO₃ crystals in 20 cc. of distilled water. Reserve part of this solution, and add to the rest ammonia, a little at a time, stirring constantly, until a sepia precipitate is thrown down, and then disappears anew. Now add the reserved solution, fractioned, until there is a slight turbidity, persisting during agitation. This reagent sheltered from light, keeps well.

39

NOTES AND REVIEWS

Dry the specimen carefully, and cover it for 30 seconds, 2 or 3 times, according to its thickness, with Rugés solution (1 cc. of crystallized acetic acid in 100 cc. of a 2% solution of formaldehyd). This dissolves the haemoglobin. Rinse in alcohol; then pass thru flame to burn off all traces of the alcohol. Cover specimen with a solution of tannin (5 gm. of tannin and 1 gm. of glacial phenol in 100 gm. water). Heat till it steams. Let it steam 1 minute, then rinse till all trace of tannin solution is gone. Dry. Cover with the nitrate solution. Rinse and dry. All the spirochetes take an even deposit of the silver, and look uniformly thicker and extremely distinct. The pale spirochete retains all its special characteristics, showing up dark purple against a transparent background or against the light yellow background of the decolored red cells.

Enlarged photographs in Forensic Medicine.—Martin of Lyons University, France, says the most valuable information is derived from enlarging a photograph of a fire-arm wound in criminal cases. The stereoscopic view, enlarged several diameters brings out details which otherwise entirely escape notice.

Improved Staining Technic. P. del Rio-Hortega (Revista Española de Medicina y Cirugia, Barcelona. Sept. 1918, L. No. 3: and J.A.M.A., p. 1620, Nov. 9, '18) gives details of a method for histologic specimens with an ammoniacal solution of silver carbonate, prepared with lithium carbonate from silver nitrate. Histological details are said to be shown up much clearer than with the classic technics. Especially useful in amylosis and for nerve fibers and tumors.

The Amoebas infesting man. H. Aragao (Annaes Paulistas de Medicina Cirugia, S. Paulo, page 25, February 1918, 9, No. 2; and J.A.M.A., 29 December 14, 1918) mentions the increase, in cases reported for S. Pau'o within the last 6 years, from 4 to 543. Drugs do not act directly on the encysted forms, but they check the multiplication of the parasite into the encysted states. None of the drugs that act on the *E. histolytica* seem to have the slightest action on the *E. coli*. Differentiation is difficult if they are dead, especially when any epithelial cells and dead leucocytes are present. He therefore incubtes at 37° C., for from $\frac{1}{2}$ to 1 hour to restore if possible, their mobility if lost. To stain, the faeces are diluted—0.5 cc. in 2 or 3 cc. of a 0.1% solution of gentian violet in physiological solution to

which has been added 0.3% of acetic acid. This keeps the elements for several days.

A Flagellate parasite occuring in a species of Euphorbia is mentioned by J. Iturbe (Gaceta Medica de Caracas. Venezuela. August 31, 1918, 25, No. 16, page 173).

Spirochetosis, filariasis, bilharziasis, pellagra, lepra occur in Porto Rico. See page 247, 14, No. 120 Boletin de la Assoc. Medica de Puerto Rico, S. Juan, September 1918.

Preparing and Mounting Slides of Crystals. Maurice E. Parker (English Mechanic, Jan. 10, 1919) discusses methods for making permanent mounts of crystals. These mounts are suitable for low powers. Take a test tube 1/4 in. bore and 2 in. long, pour in a teaspoonful of distilled water, then add enough of the chemical to be used to make a saturated solution, i.e. so that just a small amount remains undissolved. Now take a thoroughly clean slide, place a drop of the solution on the center of it, spread the drop so it covers about 3% in. diameter, allow to dry, taking great care no dust settles on it, as dust shines up brilliantly in polarized light. To obtain large and well formed crystals dry slowly; to obtain very fine, feathery crystals dry by gentle heat. When thoroughly dry mount in Canada balsam, being very careful not to displace the delicate crystals when pressing down the cover. (This usually draws down to place if only just enough medium is used.) Remember Canada balsam dissolves some chemicals; therefore mount the same object in castor oil and label the mountant on all slides. This should always be done, otherwise in remounting valuable slides in Colleges and Societies, no one can tell what treatment they should receive. Excess of the mounting material must be well cleaned off in order to ring the slides, if castor oil is used. Shellac is preferred for this by the technician, but Canada balsam is a useful one as it mixes in well.

Some workers advise the use of alcoholic or ethereal solutions, instead of aqueous, so that smaller crystals are formed, thus allowing higher powers to be used. Some of the best chemicals to try out are found among the materials used in the photographic room hydroquinone, potassium bichromate, pyrogallic acid, sodium carbonate, sulphite of sodium, sodium borate, salol, picric acid, potassium cyanide (the last needs care as it is dangerous under certain conditions). Others are menthol, potassium chlorate from the head of a safety match, kinnate quinia, salicin, sugar, etc. Try sodium benzene sulphonate, hippuric acid, and anthracene with polarized light. Hippuric acid can be made to vary its crystal forms. If dissolved in alcohol and warmed, on drying they resemble the leaves of flowers. If breathed on during cooling they take the form of rosettes. Ortho-nitro-phenol is a complex compound of the "Ring" series and if very thin on a slide its color effects are very beautiful. Coumarin shows another type.

V. A. L.

MEASURING CARBON DIOXIDE PRODUCED BY PROTOZOA

Lund (Baltimore Meeting Am. Soc. Zool. 1918) has devised a simple procedure to determine the production of CO^2 by small organisms. A wide mouth glass-stoppered bottle is used, from the stopper of which is suspended a small flat stender dish containing the organisms. A small quantity of weak $Ba(OH)_2$ is placed on the bottom of the bottle. This absorbes the CO_2 which gets into the bottle. By proper controls the amount due to the animals can be determined.

It was found by using small quantities of some substance, as Na_2CO_3 , which would set free known quantities of CO_2 , that only about 5% of error existed in measuring the quantity of CO_2 set free by an acid from even one milligram of Na_2CO_3 . Similar accuracy is insured for the production of the organisms.

METHOD FOR DEMONSTRATING GLYCOGEN IN TISSUES

Gage (J. Comp. Neur. June, 1917) summarizes the methods used by him in his studies of the distribution of glycogen in Vertebrates.

1. Fix in alcohol (67-100%). A medium is necessary which does not dissolve the very changeable glycogen. While other agents may be used, none is so generally satisfactory.

2. Imbed either in paraffin or collodion, or the combined method may be used.

3. For staining iodin is the only reliable and satisfactory agent. An alcoholic iodin stain was found most satisfactory (95% alcohol, 150 cc.; water, 150 cc.; 10% alcoholic solution of iodine, 15 cc.; iodid

42